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(54) Title: PRE-BINDING OF RETROVIRAL VECTOR PARTICLES WITH COMPLEMENT COMPONENTS TO ENABLE THE PERFORMANCE OF HUMAN GENE THERAPY <i>IN VIVO</i> (57) Abstract This invention relates to an improvement in the use of retroviral vectors in gene therapy. The invention specifically relates to the use of C1 complement subcomponents and antibody fragments to protect retroviral vector particles from attack by primate complement systems <i>in vivo</i> . Pharmaceutical compositions containing retroviral vector particles prebound with C1 complement subcomponents, as well as gene therapy methods, are part of this invention.		

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5 PRE-BINDING OF RETROVIRAL VECTOR PARTICLES WITH
COMPLEMENT COMPONENTS TO ENABLE THE PERFORMANCE
OF HUMAN GENE THERAPY *IN VIVO*

10 BACKGROUND OF THE INVENTION

15 A. Field of the Invention

 This invention relates to an improvement in the use
of retroviral vectors in gene therapy. The invention
specifically relates to the use of C1 complement subcomponents
and antibody fragments to protect retroviral vector particles
from attack by primate complement systems *in vivo*.
Pharmaceutical compositions containing retroviral vector
particles prebound with C1 complement subcomponents, as well
as gene therapy methods, are part of this invention.

20 B. Background

 There is active research, including clinical trial
research, on treatment of disease by introduction of genetic
material into specific cells of a patient. A variety of human
diseases may be treated by therapeutic approaches that involve
stably introducing a gene into a human cell such that the gene
may be transcribed and the gene product may be produced in the
cell. Diseases amenable to treatment by this approach include
inherited diseases, particularly those diseases that are
caused by a single gene defect. Many other types of diseases,
including acquired diseases, may also be amenable to gene
therapy. Examples of these diseases include many forms of
cancer, lung disease, liver disease, and blood cell disorders.
See Miller, A.D. (1992) *Nature* 357:455-460, and Mulligan, R.C.
(1993) *Science* 260:926-932, both incorporated herein by
reference.

Delivery of the gene or genetic material into the cell is the first critical step in gene therapy treatment of disease. A variety of methods have been used experimentally. Most research has focused on the use of retroviral and adenoviral vectors for gene delivery into the cell. Retroviral vectors have the ability to stably integrate the transferred gene sequences into the chromosomal DNA of the target cell. Retroviral vectors are particularly attractive because they are very efficient in stably transducing a high percentage of target cells. Accordingly most of the approved gene therapy clinical protocols use retroviral vectors. See Miller, A.D., (1992) *supra*.

Most gene therapy protocols involve treating cells from the patient *ex vivo* and then reintroducing the cells into the patient. For many diseases, however it will be necessary to introduce the gene into the cell *in vivo*, because the target cells cannot be removed from the body and returned. *Ex vivo* treatment of large numbers of patients may also not be feasible because of the great expense of treating each patient. Development of *in vivo* gene therapy is therefore critical for the successful gene therapy treatment of many serious diseases, and is necessary for gene therapy to reach its full potential.

One of the problems with the use of retroviral gene therapy vectors *in vivo* is that many retroviruses are inactivated in the circulatory system of primates, including humans, by the complement system. This problem had precluded the use of some of the most attractive retroviral vectors, such as those derived from murine leukemia viruses, for *in vivo* gene therapy.

The mechanism of this antibody-independent inactivation of retroviruses by the human complement system has been determined. The first step is the binding of complement component C1 to the retrovirus. This binding triggers the complement cascade, which results in the eventual destruction and elimination of the virus. Complement component C1 is a 3 subunit macromolecule composed of C1q, C1r, and C1s subunits. Both the C1s and C1q subunits have

specific binding sites for the retroviral coat proteins, and both the C1s and C1q subunits of the intact C1 component must bind the retrovirus particle in order to trigger the complement cascade. It has been shown, using inactive
5 retrovirus, that prebinding with C1s blocks the subsequent activation of the complement cascade by retrovirus *in vitro*. See Bartholomew, R.M., et al. (1980) *Biochemistry* 19:2847-2853. However, Bartholomew et al. did not demonstrate that live retrovirus could be protected in a subsequent challenge
10 with primate complement. The protection of active retroviral particles from primate complement is necessary for the use of the retroviral vector particles in *in vivo* gene therapy, as described herein.

This invention uses isolated C1s and isolated C1q to
15 prebind retroviral vector particles *in vitro*. Alternatively, a fragment of an antibody reactive with the retroviral vector particles may be used to prebind the retroviral vector particle. The antibody fragment must lack the complement
20 binding site present on the Fc region of the antibody to avoid triggering the antibody-dependent complement cascade when the retroviral vector particle is subsequently introduced *in vivo*. The retroviral vector particle bound by the C1s or C1q subunit or the antibody fragments retains its biological activity and may be used in *in vivo* gene therapy protocols. Thus, the
25 present invention allows for retroviral vector particles to be used for *in vivo* gene therapy.

SUMMARY OF THE INVENTION

This invention provides for methods of masking
30 primate retroviral vector particles from the primate complement system, using the isolated complement C1 subcomponents, C1s and C1q. The formation of a retroviral vector particle:C1s complex or the formation of a retroviral vector particle:C1q complex blocks the subsequent binding
35 and/or activation of complement C1 to the retroviral vector particle *in vivo* when the complex is administered to a primate. The above complexes are formed under conditions where the retroviral vector particle maintains its biological

activity. Preferably the retroviral vector contains a heterologous gene operably linked to a portion of the vector which recombines with the genome of a primate.

5 The invention also provides methods for using antibody fragments to mask primate retroviral vector particles from the primate complement system. These antibody fragments are capable of binding retroviral vector particles and lack the complement binding regions normally present in the constant region of the antibody molecule. The formation of
10 this retroviral vector particle:antibody fragment complex blocks the subsequent binding and/or activation of complement C1 by the retroviral vector particle *in vivo* when the complex is administered to a primate. The above complexes are formed under conditions where the retroviral vector particle
15 maintains its biological activity. Preferably the retroviral vector contains a heterologous gene operably linked to a portion of the vector which recombines with the genome of a primate.

The invention also provides for pharmaceutical
20 compositions for gene therapy in primates which contain the retroviral vector particle:C1 subcomponent complexes or the retroviral vector:antibody fragment complexes, and pharmaceutically acceptable carriers. Preferably the retroviral vector contains a heterologous gene operably linked
25 to a portion of the vector which recombines with the genome of a primate. Lastly, the invention provides for methods of gene therapy in which these pharmaceutical compositions are administered to primates.

30 DESCRIPTION OF THE PREFERRED EMBODIMENT

A. Definitions

"Antibody" refers to an immunoglobulin molecule able to bind to a specific epitope on an antigen. Antibodies can be a polyclonal mixture or monoclonal. Antibodies can be
35 intact immunoglobulins derived from natural sources or from recombinant sources and can be immunoreactive portions of intact immunoglobulins. Antibodies are typically tetramers of immunoglobulin molecules. The antibodies may exist in a

variety of forms including, for example, Fv, F_{ab}, and F(ab)₂, as well as in single chains (e.g., Huston, et al., *Proc. Nat. Acad. Sci. U.S.A.*, 85:5879-5883 (1988) and Bird, et al., *Science* 242:423-426 (1988), which are incorporated herein by reference). (See generally, Hood, et al., "Immunology", Benjamin, N.Y., 2nd ed. (1984), and Hunkapiller and Hood, *Nature*, 323:15-16 (1986), which are incorporated herein by reference). Single-chain antibodies, in which genes for a heavy chain and a light chain are combined into a single coding sequence, may also be used.

The term "antibody fragment" refers to any polypeptide fragment of an antibody which contains less than the entire amino acid sequence of the antibody and which retains antigen binding activity. Such fragments may be produced by any method known to those of skill in the art or as described herein.

The term "biological activity" refers to an activity of a molecule, group of molecules or an organism that has a biological function. For example, the biological activity of a retroviral vector particle is the transduction of appropriate target cells.

The term "complement system" means the complex group of interacting proteins found in vertebrates that interact with each other in a cascade reaction to produce many of the effector functions of humoral immunity. The complement system produces and regulates inflammation, the opsonization of foreign materials for phagocytosis, and mediates the direct cytotoxicity of foreign cells and microorganisms. The complement system is described in detail by Frank, M.M. and Fries, L.F. in W.E. Paul, editor (1989) *Fundamental Immunology, Second Edition*, Raven Press, New York, pp 679-701, which is incorporated herein by reference.

The term "primate complement system" refers to the complement system of a primate, including a human.

The terms "complement component C1", "C1 complement component", "C1 complement", "C1 complex", and "C1" as used herein all refer to the complement macromolecule described herein, containing the C1q subunits, C1s subunits, and C1r

subunits, and which initiates the classical complement pathway. These terms include all naturally occurring forms of C1 complex found physiologically and includes both activated and inactivated C1 complex. See Frank and Fries, *supra*.

5 The terms "C1q subunit", "C1s subunit" or "C1r subunit" refer to all naturally occurring forms of these subunits, either as free subunits, multimers or as a part of the C1 complex. These terms also include all the forms of C1q subunit, C1s subunit and C1r subunit present in both activated
10 and nonactivated C1 complexes.

 The terms "C1q", "C1s", and "C1r" refer to all forms of C1q subunits, C1s subunits and C1r subunits, respectively, that are not associated with the C1 complex, and to any peptide fragments thereof, whether naturally occurring or
15 created in the laboratory. The term "C1q" also encompasses the individual A, B, and C chains of the C1q subunit, any combination of these chains, or any peptide fragments thereof. The term "C1 complement subcomponent" refers to any form of C1q, C1s, or C1r as defined above.

20 The term "in an amount sufficient to block primate C1 complement activation" refers to an amount of a substance which causes a measurable inhibition of the activation of primate C1 complement. This inhibition of C1 complement activation can be caused by an inhibition of the binding of C1
25 complement to its target molecules. Alternatively, this inhibition of C1 complement activation may not cause measurable inhibition of the binding of C1 to its target molecules, but may prevent the activation of C1. Examples of such target molecules include those present on organisms,
30 viruses or viral vector particles. A measurable inhibition of complement C1 activation refers to at least a 1% inhibition of complement C1 activation when complement activation is measured *in vitro*. The blocking of C1 complement activation can be measured in a variety of ways as described herein.
35 Blocking of complement C1 activation may be measured by determining complement activation after retroviral vector particle:C1 complement subcomponent complexes or retroviral vector particle:antibody fragment complexes are incubated with

human complement *in vitro*. As described herein, there are a variety of methods of measuring complement activation that may be used.

Alternatively, the blocking of complement C1
5 activation may be measured indirectly by measuring protection of retroviral vector particles from lysis by human complement *in vitro*. See example 2 herein. In this assay, a measurable inhibition of complement C1 activation corresponds to a 1% inhibition of lysis at a final human serum dilution of 1:8
10 when at least 2000 colony forming units of retroviral vector particles are used in the assay.

The term "incubating" refers to contacting the desired molecules in a solution or in a mixture and maintaining the solution or mixture under established
15 conditions for a period of time.

The term "*in vivo*" as used herein means in the body of an animal, including a human. For example, a reaction occurring *in vivo* means that it occurs in the body of the animal.

20 "Nucleic acids", as used herein, may be DNA or RNA. Additionally, substantial nucleic acid sequence identity exists when a nucleic acid segment will hybridize, under stringent hybridization conditions, to a complement of another nucleic acid strand.

25 The term "vector" as used herein refers to a nucleic acid molecule, generally DNA, which is capable of being joined to a heterologous DNA fragment and of replicating in an appropriate host cell or which is capable of transducing a host cell. Vectors are used routinely in recombinant DNA
30 techniques. Any extrachromosomal small genome such as a plasmid, phage or virus is a potential vector. Such phage, plasmid or virus genomes may be modified by molecular biology techniques to have desired characteristics for use as a vector.

35 The term "retroviral vector" as used herein means a vector that is derived from a retrovirus. Retroviral vectors may be constructed to have the capability to insert a gene or DNA fragment into the host chromosomal genome by a

recombinational event, such that the DNA fragment may be stably expressed in the host cell. The basic design and use of retroviral vectors is described in Singer, M. and Berg, P. *Genes and Genomes*, Mill Valley, California (1991) pp. 310-314, which is incorporated herein by reference. A retrovirus is present in the RNA form in its viral capsule and forms a doublestranded DNA intermediate when it replicates in the host cell. Similarly, retroviral vectors are present in both RNA and doublestranded DNA forms, both of which forms are included in the term "retroviral vector". The term "retroviral vector" also encompasses the DNA form which contains a recombinant DNA fragment and the RNA form containing a recombinant RNA fragment.

The term "retroviral vector particle" as used herein refers to the RNA form of a retroviral vector associated with retroviral coat proteins to form a particle capable of transducing host cells. This particle is unable to replicate in the host cell, contains a recombinant RNA fragment and is capable of integrating a recombinant DNA fragment into the host cell's chromosomal genome. As described in detail herein, retroviral vector particles may be assembled by the use of packaging cell lines, which express the structural proteins of a retrovirus and which are capable of producing retrovirus-like particles containing the RNA form of the retroviral vector associated with retroviral structural proteins. Since retroviral vector particles do not replicate in the host cell, the process of uptake of the retrovirus vector particle into the host cell is termed transduction, rather than infection. The term "transducing" as used above therefore refers to the uptake of a retroviral vector particle into a host cell and the subsequent integration of retroviral vector DNA into the host genome.

The term "primate retroviral vector particle" means a retroviral vector particle capable of transducing a primate cell.

The term "retroviral vector particle complexes" as used herein refers to retroviral vector particle:C1q complexes, retroviral vector particle:C1s complexes, and

retroviral vector particle:antibody fragment complexes as described herein.

The term "heterologous gene" as used herein refers a gene which is inserted into the DNA of a vector where the gene is not a gene from the same species that the vector is derived from. For example, a heterologous gene inserted into a retroviral vector refers to any nonretroviral gene. Similarly, the term "heterologous DNA fragment" refers to a DNA fragment which is inserted into the DNA of a vector where the DNA fragment is not from the same species that the vector is derived from. For example, a heterologous DNA fragment inserted into a retroviral vector refers to any nonretroviral DNA fragment. A heterologous DNA fragment may contain a heterologous gene. The terms "heterologous gene" or "heterologous DNA fragment" include nucleic acid sequences endogenous to the species into which the retrovirus vector is transduced. When referring to retroviral vectors or retroviral vector particles, the terms "heterologous gene" or "heterologous DNA" also include the corresponding RNA sequences inserted into the RNA form of the retroviral vector or retroviral vector particles.

The term "gene therapy" as used herein refers to a method of treating disease in an animal by introducing a DNA fragment or gene into the cells of the animal. The DNA fragment or gene introduced may be from the same species as the animal being treated or it may originate from another species. The DNA fragment or gene may also be synthetically produced. The term includes introduction of genes into specific cells of the animal where the genes are subsequently expressed in the animal's cells and where the result of this gene expression is treatment of a disease. Examples of gene therapy using retroviral vectors are described in detail herein.

35 B. Introduction

The complement system is composed of a complex group of interacting blood proteins that mediate direct cytotoxicity against invading cells and microorganisms. The complement

proteins interact with each other in a regulated manner to provide the effector functions of both humoral immunity and inflammation. See Frank and Fries, *supra*. The complement system operates as a cascade reaction in which a series of complement proteins are activated. The primary complement activation pathway has been termed the classical complement pathway. The classical complement pathway requires the presence of antibodies to the foreign cell or microorganism and is the major effector mechanism for antibody-mediated immune responses. Formation of an antigen-antibody complex is recognized by complement component C1, which then triggers the complement cascade.

Complement activation may also occur via the alternative pathway. The alternative pathway is an antibody-independent pathway that involves only complement component C3 and the late acting complement components. Thus, titers of the early acting complement components, C1, C2, and C4 are not affected by alternative pathway activation. The alternative pathway plays a role in the serum bactericidal reaction, in viral neutralization and in the acid lysis of erythrocytes from patients with paroxysmal nocturnal hemoglobinuria. See Frank and Fries, *supra*.

Many retroviruses can activate the classical complement pathway in primates by a unique antibody-independent mechanism. This antibody-independent mechanism is found in primates and is generally not present in other mammals. See Cooper, N.R., et al. (1976) *J. Exp. Med.* 144:970-984. By this mechanism, complement component C1 binds the retrovirus particle directly and triggers the classical complement pathway, just as normally happens in the presence of an antigen-antibody complex.

Complement component C1 is a large complex protein composed of 3 subunits designated C1q, C1s, and C1r. C1q is itself composed of 18 polypeptide chains of three different types designated A, B, and C. Six molecules each of chains A, B, and C compose the C1q subunit. There are two molecules each of the C1s subunit and the C1r subunit that associate

with C1q to form the C1 complement component. See Frank and Fries, *supra*.

The C1q subunit contains multiple identical binding sites for the complement binding regions of immunoglobulin molecules. In the classical pathway, the binding of C1q to these regions of the antibody molecules of the antigen-antibody complex, causes a conformational change in the C1 complex resulting in the enzymatic activation of C1 to an active serine protease. The C1s and C1q subunits both have a molecular weight of approximately 85 kD which is cleaved to smaller molecular weight forms of approximately 57 kD and 28 kD during activation of the C1 complex. The 57 kD forms of the C1s and C1q present in the activated C1 complex contain the protease activity.

In the antibody-independent activation of the classical complement pathway by retroviruses, the C1q subunit of C1 binds to a binding site(s) on the retrovirus. In the case of Moloney murine leukemia virus, the p15E viral protein has been identified as the C1 binding receptor. See Bartholomew, R.M. et al. (1978) *J. Exp. Med.* 147:844-853. In contrast to the antibody-mediated classical complement pathway, binding by both the C1q subunit and the C1s subunit of the C1 complex is required for complement activation. Furthermore, the C1s subunit and C1q subunit must bind the virus when they are present in a functional C1 complex in order for complement activation to occur by this mechanism. See Bartholomew, et al. (1980) *Biochemistry* 19:2847-2853.

The present invention uses free C1q or free C1s to block the subsequent binding and/or activation of the C1 complex by retrovirus particles or by retroviral vector particles. As described in detail below, C1s or C1q or a combination thereof may be incubated with the retroviral vector particle *in vitro* to form a complex with the retroviral vector particle. This complex formation protects the retroviral vector particle from subsequent inactivation or lysis when the retroviral vector particle is exposed to the primate complement system, since the binding sites for C1s and/or C1q are blocked. Alternatively, one may use antibodies

to block the inactivation or lysis of the retroviral vector particles by primate complement. Antibodies are generated that bind the viral capsid proteins of the retroviral vector particles and which also block the subsequent binding and/or activation of the primate C1 complex. Fragments of these antibodies which retain binding activity for the retroviral vector particles, but which lack the complement binding regions are used in this approach to avoid activating the antibody-dependent complement pathway.

10

C. Preparation of Retroviral Vector Particles

1. Retroviral Vectors

Retroviral vectors are particularly useful for modifying mammalian cells, including primate cells, because of the high efficiency with which the retroviral vectors transduce target cells and integrate into the target cell genome. Additionally, retroviral vectors are highly useful because the vectors may be based on retroviruses that are capable of infecting mammalian cells from a wide variety of species and tissues.

20

Retroviral vectors are produced by genetically manipulating retroviruses. Retroviruses are RNA viruses; that is, the viral genome is RNA. The genomic RNA is, however, reverse transcribed into a DNA copy which is integrated stably and efficiently into the chromosomal DNA of transduced cells. This stably integrated DNA copy is referred to as a provirus and is inherited by daughter cells as is any other gene. The wild type retroviral genome and the proviral DNA have three genes: the *gag*, the *pol* and the *env* genes, which are flanked by two long terminal repeat (LTR) sequences. The *gag* gene encodes the internal structural (nucleocapsid) proteins; the *pol* gene encodes the RNA directed DNA polymerase (reverse transcriptase); and the *env* gene encodes viral envelope glycoproteins. The 5' and 3' LTRs serve to promote transcription and polyadenylation of virion RNAs. Adjacent to the 5' LTR are sequences necessary for reverse transcription of the genome (the tRNA primer binding site) and for efficient encapsulation of viral RNA into particles (the Psi site). See

35

Mulligan, R.C., In: *Experimental Manipulation of Gene Expression*, M. Inouye (ed), 155-173 (1983); Mann, R., et al., *Cell*, 33:153-159 (1983); Cone, R.D. and R.C. Mulligan, *Proceedings of the National Academy of Sciences, U.S.A.*,
5 81:6349-6353 (1984).

The design of retroviral vectors is well known to one of skill in the art. See Singer, M. and Berg, P. *supra*. In brief, if the sequences necessary for encapsidation (or packaging of retroviral RNA into infectious virions) are
10 missing from the viral genome, the result is a *cis* acting defect which prevents encapsidation of genomic RNA. However, the resulting mutant is still capable of directing the synthesis of all virion proteins. Retroviral genomes from which these sequences have been deleted, as well as cell lines
15 containing the mutant genome stably integrated into the chromosome are well known in the art and are used to construct retroviral vectors. Preparation of retroviral vectors and their uses are described in many publications including European Patent Application EPA 0 178 220, U.S. Patent
20 4,405,712, Gilboa, *Biotechniques* 4:504-512 (1986), Mann, et al., *Cell* 33:153-159 (1983), Cone and Mulligan, *Proc. Natl. Acad. Sci. USA* 81:6349-6353 (1984), Eglitis, M.A, et al. (1988) *Biotechniques* 6:608-614, Miller, A.D. et al. (1989) *Biotechniques* 7:981-990, Miller, A.D. (1992) *Nature*, *supra*,
25 Mulligan, R.C. (1993), *supra*. and Gould, B. et al., and International Patent Application No. WO 92/07943 entitled "Retroviral Vectors Useful in Gene Therapy". The teachings of these patents and publications are incorporated herein by reference.

30

2. Heterologous Genes for Retroviral Vectors

A variety of genes and DNA fragments are of interest for insertion into retroviral vectors for use in gene therapy. These DNA fragments and genes encode proteins of use in gene
35 therapy. Proteins of interest in gene therapy include various hormones, growth factors, enzymes, lymphokines, cytokines, receptors and the like. Of particular interest for use as genes for expression are those genes encoding polypeptides

either absent, produced in diminished quantities, or produced in mutant form in individuals suffering from a genetic disease. Additionally, it is of interest to use foreign genes encoding polypeptides for secretion from the target cell so as to provide for a systemic effect by the protein encoded by the foreign gene.

For example, retroviral vectors may be used for gene therapy to treat congenital genetic diseases, acquired genetic diseases (e.g., cancer), viral diseases (e.g., AIDS, mononucleosis, herpes virus infection, cytomegalovirus infection, papillomavirus infection) or to modify the genome of selected types of cells of a patient for any therapeutic benefit. Treatable disorders include hemophilia, thalassemias, ADA deficiency, familial hypercholesterolemia, inherited emphysema, cystic fibrosis, Duchenne's muscular dystrophy, lysosomal storage diseases, Gaucher's disease, and chronic granulomatous disease.

Specific heterologous genes of interest include those encoding hemoglobin, ADA, interleukins, GM-CSF, G-CSF, M-CSF, human growth factor, insulin, factor VIII, factor IX, tPA, LDL receptors, tumor necrosis factor, PDGF, EGF, NGF, IL-1ra, EPO, β -globin and the like, as well as biologically active muteins of these proteins. Genes for expression for insertion into retroviral vectors may be from a variety of species; however, preferred species sources for genes of interest are those species into which the retroviral vector containing the foreign gene of interest is to be transduced.

The retroviral vectors used in the subject invention find a variety of uses in the treatment of various medical conditions including, without limitation, cancer, genetically based diseases, cardiopulmonary diseases, endocrinological diseases, and the like. See Miller, A.D. (1992) *supra* and Mulligan (1993) *supra*.

Retroviral vector constructs which contain heterologous genes or heterologous DNA fragments may be prepared by a variety of methods known to those of skill in the art, using the retroviral vectors described above. See Sambrook, T., et al., *Molecular Cloning - A Laboratory Manual*

(2nd Ed.) Vol. 1-3, Cold Spring Harbor Laboratory, Cold Spring Harbor (1989) incorporated herein by reference, and Miller, A.D., et al. (1989) *supra* and Eglitis, M.A. (1988), *supra*.

5 3. Retroviral Vector Particles

 The retroviral vector particles are prepared by genetically altering a retrovirus vector and packaging the vector with retroviral capsid proteins by use of a packaging cell line as described above. The resultant retroviral vector
10 particle is incapable of replication in the host cell and is capable of integrating a heterologous DNA fragment into the host cell's genome. The heterologous DNA fragment may contain genes suitable for treatment of variety of diseases by gene therapy.

15 Packaging cell lines are used to prepare the retroviral vector particles. A packaging cell line is a genetically constructed mammalian tissue culture cell line that produces the necessary viral structural proteins required for packaging, but which is incapable of producing infectious
20 virions. Retroviral vectors, on the other hand, lack the structural genes but have the nucleic acid sequences necessary for packaging. Thus, transfecting retroviral vectors into packaging cell lines, either with or without the heterologous genes for expression inserted into the vector insertion site,
25 results in the production of retroviral vector particles with the desired genetic construction. See Miller, A.D. (1990), *Human Gene Ther.* 1:5-14, which is incorporated herein by reference.

 To prepare a packaging cell line, an infectious
30 clone of a desired retrovirus, in which the packaging site has been deleted, is constructed. Cells comprising this construct will express all structural proteins but the introduced DNA will be incapable of being packaged. Alternatively, packaging cell lines can be produced by transforming a cell line with
35 one or more expression plasmids encoding the appropriate core and envelope proteins. In these cells, the *gag*, *pol*, and *env* genes can be derived from the same or different retroviruses.

A number of packaging cell lines suitable for the present invention are also available in the prior art. Examples of these cell lines include Crip, GPE86, PA317 and PG13. See Miller et al., *J. Virol.* 65:2220-2224 (1991), which is incorporated herein by reference. Examples of other packaging cell lines are described in Cone, R. and Mulligan, R.C., *Proceedings of the National Academy of Sciences, USA*, 81:6349-6353 (1984) and in Danos, O. and R.C. Mulligan, *Proceedings of the National Academy of Sciences, USA*, 85: 6460-6464 (1988), Eglitis, M.A., et al. (1988) *supra* and Miller, A.D., (1990) *supra*, also all incorporated herein by reference. The GPE86 packaging cell line is described in Markowitz, N. et al. (1988) *J. Virol.* 62:1120-1124, and its use with the LXCX vector described in Miller, A.D., et al. (1989) *supra*, is demonstrated in Example 1 herein.

Packaging cell lines capable of producing retroviral vector particles with chimeric envelope proteins may be used. Alternatively, amphotropic or xenotropic envelope proteins, such as those produced by PA317 and GPX packaging cell lines may be used to package the retroviral vectors.

D. Preparation of C1s, C1q

Human C1s and C1q can be purified from human blood, sera or plasma using procedures known to those of skill in the art. For example, human C1q may be purified as described in Yonemasu, K., et al. (1971) *J. Immunol.* 106:304-313 and human C1s may be purified as described in Valet, G., et al. (1974) *J. Immunol.* 112:339-350.

Alternatively, C1q and C1s may be purchased commercially from sources known to those of skill in the art. For example, C1q may be obtained from Calbiochem and C1s may be obtained from New England Immunology Associates as described in example 2.

F. Preparation of Antibody Fragments

1. Production of Antibodies

Antibodies to the retroviral vector particle coat proteins may be produced by a variety of methods known to

those of skill in the art. For example, the retrovirus, the retroviral vector particle, or individual retroviral capsid proteins may be used for production of antibodies. Peptides of the retroviral capsid proteins, including synthetic peptides may also be used. Preferably, individual peptide regions associated with complement C1 binding to the retrovirus or the retroviral vector particle are used. Amino acid sequences which are known to be involved in C1 complement binding or which are suspected of being involved in such binding may be synthesized. For example, peptide sequences in the Moloney murine leukemia virus protein p15E which are the putative C1q binding site are identified and a number of short peptides are synthesized for use in antibody production. Preferably, the following two p15E peptides are synthesized:

15 NH₂-AVQDDLREVEKSISNLEKSL-COOH and NH₂-RDSMAKLRERLNQRQKLFES-COOH (Seq I.D. No. 1 and Seq. I.D. No.2, respectively).

The above peptides can be synthetically prepared in a variety of ways. For instance, polypeptides of relatively short size can be synthesized in solution or on a solid support in accordance with conventional techniques. Various automatic synthesizers are commercially available and can be used in accordance with known protocols. See, for example, Stewart and Young, *Solid Phase Peptide Synthesis*, 2d. ed., Pierce Chemical Co. (1984). Peptides synthesized as described above are conjugated to proteins, larger polypeptides or polymers for the purposes of immunization and antibody generation. See Harlow and Lane, *supra*.

Either monoclonal or polyclonal antibodies may be generated. Methods of production of polyclonal antibodies are known to those of skill in the art. In brief, an immunogen, preferably a purified protein, is mixed with an adjuvant and animals are immunized. The animal's immune response to the immunogen preparation is monitored by taking test bleeds and determining the titer of reactivity to the immunogen. When appropriately high titers of antibody to the immunogen are obtained, blood is collected from the animal and antisera are prepared. Further fractionation of the antisera to enrich for

antibodies reactive to the immunogen can be done if desired.
(See Harlow and Lane, *supra*.)

Monoclonal antibodies may be obtained by various techniques familiar to those skilled in the art. Briefly,
5 spleen cells from an animal immunized with a desired antigen are immortalized, commonly by fusion with a myeloma cell (See, Kohler and Milstein, *Eur. J. Immunol.* 6:511-519 (1976), incorporated herein by reference). Alternative methods of immortalization include transformation with Epstein Barr
10 Virus, oncogenes, or retroviruses, or other methods well known in the art. Colonies arising from single immortalized cells are screened for production of antibodies of the desired specificity and affinity for the antigen, and yield of the monoclonal antibodies produced by such cells may be enhanced
15 by various techniques, including injection into the peritoneal cavity of a vertebrate host.

Alternatively, one may isolate DNA sequences which code for a monoclonal antibody or portion thereof that specifically binds to the retroviral capsid proteins according
20 to methods generally known to those of skill in the art. For example, this may be done by screening a DNA library from human B cells according to the general protocol outlined by Huse et al., *Science* 246:1275-1281 (1989), and described in WO 90/14430, incorporated herein by reference, and then cloning
25 and amplifying the sequences which encode the antibody (or binding fragment) of the desired specificity.

2. Production of Antibody Fragments

Antibody fragments are produced that both retain
30 their ability to bind to retroviruses or retroviral vector particles and which lack the complement binding region present on the Fc region of the immunoglobulin molecule. A variety of types of fragments meeting this criteria may be produced.

An F(ab')₂ fragment lacks the C-terminal portion of
35 the heavy chain constant region, and has a molecular weight of approximately 110 kD. It retains the two antigen binding sites and the interchain disulfide bonds in the hinge region, but it does not have the effector functions of an intact IgG

molecule. An $F(ab')_2$ fragment may be obtained from an IgG molecule by proteolytic digestion with pepsin at pH 3.0-3.5 using standard methods such as those described in Harlow and Lane, *supra*.

5 An F_{ab} fragment comprises a light chain and the N-terminus portion of the heavy chain to which it is linked by disulfide bonds. It has a molecular weight of approximately 50 kD and contains a single antigen binding site. F_{ab} fragments may be obtained from $F(ab')_2$ fragments by limited
10 reduction, or from whole antibody by digestion with papain in the presence of reducing agents. (See, Harlow and Lane, *supra*.) In certain cases, the concentration of reducing agent necessary to maintain the activity of papain in the presence
15 of atmospheric oxygen is sufficient to fully reduce the interchain disulfide bonds to the antibody. This can result in loss of antigen recognition. To circumvent this problem, papain may be activated and then exchanged into buffer containing a concentration of reducing agent compatible with
20 maintaining antigen binding activity. The antibody digestion is carried out under an inert atmosphere to prevent deactivation of the papain.

 Antigen binding regions (e.g. the $F(ab')_2$, variable or hypervariable (complementarity determining) regions), of monoclonal antibodies may also be made using recombinant DNA
25 techniques. Such methods are generally known in the art and are described in, for example, U.S. 4,816,397, EP publications 173,494 and 239,400, which are incorporated herein by reference.

30 3. Selection of Antibody Fragments for use in Gene Therapy

 Antibodies produced as described above are characterized for their ability to block the binding and/or
activation of primate C1 complement by retroviruses or
35 retroviral vector particles. C1 complement binding may be measured directly or indirectly. Indirect means of measuring C1 complement binding includes a variety of assays for measuring complement activation. Because C1 complement can

also bind the constant region of the antibody molecule and activate the antibody-dependent classical complement pathway, it is preferable to use antibody fragments lacking the complement binding region in these measurements.

5 a. Direct Measurement of C1 Complement Binding

 The binding of C1 complement to retroviral vector particles may be measured in a variety of binding assays known to those of skill in the art. For example, isolated C1 complex may be radiolabeled and incubated with the retroviral
10 vector particles. Free and bound C1 are then separated and the radioactivity associated with the retroviral particles is determined. F_{ab} fragments, for example, from candidate antibodies are preincubated with the viral particles in order to determine their ability to block the binding of the
15 radioactive C1.

 A variety of binding assay formats may be used to measure C1 binding to the retroviral vector particles. Examples of different binding assay formats are described in detail for immunoassays. See, for example, *Enzyme*
20 *Immunoassay*, E.T. Maggio, ed., CRC Press, Boca Raton, Florida (1980); "Practice and Theory of Enzyme Immunoassays," P. Tijssen, *Laboratory Techniques in Biochemistry and Molecular Biology*, Elsevier Science Publishers B.V. Amsterdam (1985); and, Harlow and Lane, *Antibodies, A Laboratory Manual*, Cold
25 Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1989), each of which is incorporated herein by reference. The assay formats described in these references may be readily adapted by one of skill in the art to measure the binding of C1 to retroviral particles.

30 b. Measurement of Complement Activation

 Complement activation may be measured by a variety of techniques known to those of skill in the art. Some antibody fragments may not block the binding of C1 complement to retroviral vector particles, but may block the C1
35 complement activation. These antibody fragments would be selected in a complement activation assay, but would not be selected in an assay measuring inhibition of C1 complement binding. As an example of a complement activation assay, the

C1s and C1r subunits of the C1 complex may be radiolabeled and C1 activation may be measured by the conversion of the C1s and C1r zymogens detected on SDS gel electrophoresis. See Bartholomew, R.M., and Esser, A.F.(1980), *supra*.

5 c. Protection from Primate Complement

Alternatively, the antibody fragments may be selected by their ability to protect retrovirus or retroviral vector particles from a challenge by primate complement. This type of assay would also detect those antibody fragments which
10 do not block binding of C1 complement but rather interfere with the activation of the bound C1 complement. A variety of such assays are known to those of skill in the art. For example, the biological activity of a retroviral vector particle may be assayed as described in example 3 herein.
15 Alternatively, assays for lysis of retrovirus or retroviral particles may be used. See for example, Welsh R. M., et al. (1975) *Nature* 257:612-614 and Welsh, R. M., et al. (1976) *Virology* 74:432-440.

20 G. Preparation of Retroviral Vector Particle Complexes

1. Retroviral Vector Particle:C1 Complement Subcomponent Complexes

Retroviral vector particle:C1s complexes and retroviral vector particle:C1q complexes may be formed by
25 incubating the retroviral vector particles with C1s or C1q in a variety of buffer solutions containing approximately physiological levels of saline or salts at temperatures ranging from approximately 25°C to 37°C. The length of the incubation will vary according to the temperature chosen and
30 the concentrations of retroviral vector particles and C1q or C1s that are used. The concentrations of retroviral vector particles used will vary according to the desired therapeutic dose selected. See below. The concentration of C1q or C1s used will vary with the amount of retroviral vector particles
35 that are used. For an example of incubation conditions suitable for forming retroviral vector particle:C1q and retroviral vector particle:C1s complexes, see example 3 herein.

Alternatively, the incubation conditions are optimized by measuring the inhibition of primate C1 binding to the retroviral vector particles in the complex. C1 binding may be measured directly or indirectly by measuring complement activation using the procedures described herein.

2. Retroviral Vector Particle:Antibody Fragment Complexes

Retroviral vector particles prepared as described above are incubated with the antibody fragments prepared as described above which are capable of blocking the binding of complement C1 to the retroviral vector particles or the subsequent activation of C1. Retroviral vector particle:antibody fragment complexes may be formed by incubating the retroviral vector particles with the selected antibody fragment or fragments in a variety of buffer solutions containing approximately physiological levels of saline or salts at temperatures ranging from approximately 25°C to 37°C. The length of the incubation will vary according to the temperature chosen and the concentrations of retroviral vector particles and the antibody fragments that are used. The concentrations of retroviral vector particles used will vary according to the desired therapeutic dose selected. See below. The concentration of antibody fragment used will vary with the amount of retroviral vector particles that are used. For example the retroviral vector particles may be incubated with the antibody fragments in PBS at room temperature similar to the method for formation of the retroviral vector particle: C1 complement subcomponent complexes that is described in example 3, herein.

Alternatively, the formation of retroviral vector particle:antibody fragment complex may be measured for its inability to bind primate C1 complement. The conditions for preparation of the complexes are then optimized by measuring C1 complement binding or complement activation as described herein.

H. Testing the Retroviral Vector Particle Complexes

The retroviral vector particle complexes formed as described above are tested for biological activity when challenged with primate complement. After incubation with
5 primate serum *in vitro*, the retroviral vector particles can be titered on an appropriate cell line. Markers such as neomycin resistance incorporated into the vector may used to detect the retroviral particles. See Eglitis, M. A., et al. (1985) *Science* 230:1395-1398 and example 2, herein. Other markers
10 such as β -glucuronidase, β -galactosidase, thymidine kinase, dihydrofolate reductase, xanthine-guanine phosphoribosyltransferase, CAD, hygromycin B kinase, adenosine deaminase, puromycin N-acetyl transferase, multidrug resistance P-glycoprotein, tryptophan synthetase, histidinol
15 dehydrogenase, or pheomycin binding protein may be used instead of neomycin. See Eglitis, M.A. (1991) *Human Gene Therapy* 2:195-201.

I. Pharmaceutical Compositions and Administration of Gene Therapy

As described herein, retroviral vectors are particularly suitable for delivering heterologous genes to cells for gene therapy of a number of diseases. Current strategies for gene therapy are reviewed in Miller A.D, (1992)
25 *supra* and Mulligan, R.C., *supra*.

The retroviral vector particle complexes of the invention can be used to introduce DNA fragments into a variety of cells and tissues including myeloid cells, bone marrow cells, lymphocytes, hepatocytes, fibroblasts, lung
30 cells, and muscle cells. For example, DNA fragments containing genes conferring resistance to a chemotherapeutic agent may be transferred to non-neoplastic cells, especially hematopoietic cells. Alternatively, DNA fragments comprising a toxin gene (e.g., ricin or diphtheria toxin) expression
35 cassette or a negative selectable marker gene expression cassette may be selectively inserted into neoplastic cells. Expression of the toxin gene or negative selection gene (followed by negative selection) selectively kills target

cells. DNA fragments which are not cytotoxic but which reverse or suppress the neoplastic phenotype (e.g. antisense inhibition of oncogene expression) also may be used to treat cancer, as well. Other uses include the introduction of immunomodifiers into bone marrow cells to treat cancers.

Delivery of the DNA fragment of interest may be accomplished *in vivo* by administration of the retroviral vector particle complexes to an individual patient, typically by systemic administration (e.g., intravenous, intraperitoneal, intramuscular, subdermal, or intracranial infusion).

The retroviral vector particle complexes of the present invention are useful in the treatment of a variety of immune system diseases. For such treatment, the retroviral vector particle complexes can be formulated for a variety of modes of administration, including systemic and localized administration.

For systemic administration, injection is preferred, including intramuscular, intravenous, intraperitoneal, and subcutaneous injection. Suitable formulations for injection are found in *Remington's Pharmaceutical Sciences*, Mack Publishing Company, Philadelphia, PA, 17th ed. (1985), which is incorporated herein by reference. A variety of pharmaceutical compositions comprising complexes of the present invention and pharmaceutically effective carriers can be prepared. The pharmaceutical compositions are suitable in a variety of drug delivery systems. For a brief review of present methods of drug delivery, See, Langer, *Science* 249:1527-1533 (1990) which is incorporated herein by reference.

The retroviral vector particle complexes of the present invention may be prepared as formulations in pharmaceutically acceptable media, for example, saline, phosphate buffered saline, Hank's solution, Ringer's solution, dextrose/saline, glucose solutions and the like. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions, such as buffering agents, tonicity adjusting

agents, wetting agents, and the like. Additives may also include additional active ingredients such as bactericidal agents, or stabilizers.

5 Systemic administration can also be by transmucosal or transdermal means, or the conjugates can be administered orally. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrations are generally known in the art, and include, for example, for transmucosal
10 administration, bile salts and fusidic acid derivatives. Transmucosal administration may be through nasal sprays, for example, or using suppositories.

 In preparing pharmaceutical compositions of the present invention, it may be desirable to modify the complexes
15 of the present invention to alter their pharmacokinetics and biodistribution. For a general discussion of pharmacokinetics, See, *Remington's Pharmaceutical Sciences*, supra, Chapters 37-39. A number of methods for altering pharmacokinetics and biodistribution are known to one of
20 ordinary skill in the art (See, e.g., Langer, supra). Examples of such methods include protection of the complexes in vesicles composed of substances such as proteins, lipids (for example, liposomes), carbohydrates, or synthetic polymers.

25 For example, the complexes of the present invention may be incorporated into liposomes in order to enhance their pharmacokinetics and biodistribution characteristics. A variety of methods are available for preparing liposomes, as described in, e.g., Szoka et al., *Ann. Rev. Biophys. Bioeng.*
30 9:467 (1980), U.S. Pat. Nos. 4, 235,871, 4,501,728 and 4,837,028, all of which are incorporated herein by reference. Following the above treatment, the liposome suspension is brought to a desired concentration for use in intravenous administration. The suspension is then sterilized by
35 filtration and the liposomes may be administered parenterally or locally in a dose which varies according to, e.g., the manner of administration, the drug being delivered, the particular disease being treated, etc.

For pharmaceutical compositions which comprise the complexes of the present invention, the dose will vary according to, e.g., the particular complex, the manner of administration, the particular disease being treated and its severity, the overall health and condition and age of the patient, and the judgment of the prescribing physician. Dosage levels for human subjects are generally between about 10^6 and 10^{14} colony forming units of retroviral vector particle per patient per treatment.

The pharmaceutical compositions are intended for parenteral or local administration, such as by aerosol or transdermally, for prophylactic and/or therapeutic treatment. The pharmaceutical compositions can be administered in a variety of unit dosage forms depending upon the method of administration.

The pharmaceutical compositions may be administered intravenously. Thus, this invention provides compositions for intravenous administration which comprise a solution of the complex dissolved or suspended in an acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers may be used, e.g., water, buffered water, 0.4% saline, and the like. For instance, phosphate buffered saline (PBS) is particularly suitable for administration of the complexes of the present invention. These compositions may be sterilized by conventional, well-known sterilization techniques, or may be sterile filtered. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions, such as pH adjusting and buffering agents, tonicity adjusting agents, wetting agents and the like, for example, sodium acetate, sodium lactate, sodium chloride, potassium chloride, calcium chloride, sorbitan monolaurate, triethanolamine oleate, etc.

For aerosol administration, the complexes are preferably supplied in finely divided form along with a surfactant and propellant. The surfactant must, of course, be nontoxic, and preferably soluble in the propellant. Representative of such agents are the esters or partial esters of fatty acids containing from 6 to 22 carbon atoms, such as

caproic, octanoic, lauric, palmitic, stearic, linoleic, linolenic, olesteric and oleic acids with an aliphatic polyhydric alcohol or its cyclic anhydride such as, for example, ethylene glycol, glycerol, erythritol, arabitol, mannitol, sorbitol, the hexitol anhydrides derived from sorbitol, and the polyoxyethylene and polyoxypropylene derivatives of these esters. Mixed esters, such as mixed or natural glycerides may be employed. The surfactant may constitute 0.1%-20% by weight of the composition, preferably 0.25-5%. The balance of the composition is ordinarily propellant. Liquefied propellants are typically gases at ambient conditions, and are condensed under pressure. Among suitable liquefied propellants are the lower alkanes containing up to 5 carbons, such as butane and propane; and preferably fluorinated or fluorochlorinated alkanes. Mixtures of the above may also be employed. In producing the aerosol, a container equipped with a suitable valve is filled with the appropriate propellant, containing the finely divided compounds and surfactant. The ingredients are thus maintained at an elevated pressure until released by action of the valve. See *Remington's Pharmaceutical Sciences, supra*.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned hereunder are incorporated herein by reference. Unless mentioned otherwise, the techniques employed or contemplated herein are standard methodologies well known to one of ordinary skill in the art. The materials, methods and examples are illustrative only and not limiting.

EXAMPLES

1. Production of Retroviral Vector Particles

The GPE86 packaging cell line is prepared as described by Markowitz, D., et al., *supra*. The GPE86 cells are grown in DMEM cell culture medium supplemented with 2 mM glutamine and 10% heat-inactivated fetal calf serum. The cells were transduced with the LNCX retroviral vector to generate the producer cell population, GPE86-LNCX. See Miller, A.D., et al., (1989) *supra*. Supernatant is collected from the GPE86-LNCX cells and diluted in the DMEM medium supplemented with 2mM glutamine and 10% fetal calf serum to a neomycin resistance titer of approximately 2×10^{-4} colony forming units/ml on TK⁻ 3T3 cells. See, Eglitis, M.A., et al., (1985) *supra*. This stock is used for all subsequent experiments.

2. Complement Resistance Titer Assay

Human serum is prepared by first collecting 150 ml of whole blood from various donors in red top clot tubes. The blood is allowed to clot for one hour at room temperature. The tubes are then spun at 2500 rpm for 10 minutes in a clinical centrifuge. The serum is then stored in 10 ml aliquots at -70°C. Heat inactivation of control aliquots to destroy complement activity is performed at 56°C for 30 minutes.

Retroviral vector particles are then preincubated with C1s, C1q, or F_{ab} antibody fragments. Human C1q was obtained from Calbiochem at 1-1.2 mg/ml, and stored frozen at -70°C until use. The C1q is then diluted into half physiological ionic strength PBS pH 7.2 (1/2X PBS). Human C1s obtained from New England Immunology Associates, is dissolved into 1/2X PBS containing 0.15 mM CaCl₂ and 0.5 mM MgCl₂, in order to maintain C1s in dimeric form. 100 µl of the retroviral vector particles, prepared as described above, are added to 100 µl of various concentrations of C1q in 1/2X PBS or C1s in 1/2X PBS, 0.15 mM CaCl₂, 0.5mM MgCl₂. The mixtures are incubated for 60 minutes at room temperature.

Following the preincubation with C1s or C1q, the vector particles are then challenged for 30 minutes at 37°C with an equal volume of human serum prepared as described above. Heat-treated human serum also prepared as described above is used as a control. Vector particles are then titered for neomycin resistance on TK⁻ 3T3 cells. See, Eglitis, M.A., et al. (1985) *supra*.

The percentage of retroviral vector particle activity protected at each serum dilution is calculated as follows:

$$\% P = \frac{\text{CFU (+ C1Q + fresh serum)}}{\text{CFU (+ C1Q + heat inact. serum)}} \times 100 \text{ minus } \% \text{ Bkgd}$$

$$\text{where } \% \text{ Bkgd} = \frac{\text{CFU (+ fresh serum)}}{\text{CFU (+ heat inact. serum)}} \times 100$$

Thus, background survival refers to the percent survival of the retroviral vector particles upon challenge with human serum. Detectable background survival of vector particles only occurs under these experimental conditions at large dilutions of serum which have less complement available to lyse vector particles. Any such background survival is subtracted out according to the above equation.

3. Protection of Retroviral Vector Particle Activity by Prebinding with C1q

There are three main variables that characterize the protection of retroviral vector particle activity by C1q from inactivation by human complement. They are the 1) the amount of retroviral vector particles used, 2) the concentration or amount of C1q used, and 3) the amount or dilution of human serum (complement) used to challenge the retroviral vector particles preincubated with C1q. The interdependence of these variables is illustrated in the following experiments.

Initially, 6700 colony forming units of the GPE86-LNCX retroviral vector particles were incubated with 12 µg and 120 µg of C1q, and then subsequently challenged with fresh human serum at final dilutions of 1/4 and 1/8 under the

conditions described above. At 12 μ g of Clq, no activity remained when the retroviral particles assayed for neomycin-resistant colonies on Tk- 3T3 cells. However, at 120 μ g of Clq, 1.6% of the vector particles survived at the 1/4 dilution of serum and 8.1% survived at the 1/8 dilution of serum. No background survival of the retroviral vector particles were observed at these serum dilutions.

The percentage of virolytic activity for retroviruses remaining at various dilutions of human serum was determined in Sherwin, et al., (1978) *Int. J. of Cancer* 21:6-11. At 1/4 and 1/8 final concentrations of human serum, there was approximately 90% and 50% of virolytic activity remaining, respectively. Therefore, under conditions where 90% of human complement activity remains (a 1/4 final serum dilution), 1.6% of the retroviral vector particles are protected in the above experiment. By scaling up proportionally the number of retroviral vector particles and the amount of Clq added, the same percentage of retroviral vector particles protected may be obtained with a much higher number of retroviral vector particles. For example, at 1% protection of retroviral vector particles, one could start with 10^{10} retroviral vector particles and obtain 10^8 protected retroviral vector particles. This represents a workable number of protected retroviral vector particles for gene *in vivo* gene therapy. Thus, the present invention makes feasible the use of retroviral vector particles in *in vivo* gene therapy in primates by protecting the retroviral vector particles from lysis by primate complement *in vivo*.

Subsequently, 2000 colony forming units of the GPE86-LNCX retroviral vector particles were incubated with 120 μ g of Clq at various serum dilutions in the above protocol. Protection of 50% of the retroviral vector particles at these concentrations was achieved at a serum dilution of 1/20, thus further demonstrating the efficacy of Clq to protect the retroviral vector particles from destruction by human complement.

4. Protection of Retroviral Vector Particle Activity by Prebinding with C1s

Approximately 3100 colony forming units of retroviral vector particles were incubated with 100 μ g of C1s and then challenged with human serum as described above. Human serum dilutions of 1/8, 1/16, and 1/32 were used. The controls (without preincubation with C1s) showed no survival of the retroviral vector particles at the 1/8 and 1/16 dilutions and only a 0.7% survival at the 1/32 dilution of the human serum. Any such background was subtracted from the percentage of retroviral vector particles protected as described above. Results of the experiment are shown in the table below.

15

TABLE 1

Protection of Retroviral Vector Particle Activity
By Prebinding with C1s

	<u>Serum dilution</u>	<u>Percent Protection</u>	<u>Background</u>
	1/8	10.8	0.0
20	1/16	26.2	0.0
	1/32	99.3	0.7

According to Sherwin, et al., (1978) *supra*, at a 1/8 final concentration of human serum, there is approximately 50% of the virolytic activity of human serum remaining. Therefore, under conditions where 50% of human complement activity remains (a 1/8 final serum dilution), 10.8% of the retroviral vector particles are protected in the above experiment. By scaling up proportionally the number of retroviral vector particles and the amount of C1s added, the same percentage of retroviral vector particles protected may be obtained with a much higher number of retroviral vector particles. For example, at 5% protection of retroviral vector particles, one could start with 10^{10} retroviral vector particles and obtain 5×10^8 protected retroviral vector particles. As described above, this represents a workable number of protected retroviral vector particles for gene in vivo gene therapy. Thus, the present invention makes feasible

the use of retroviral vector particles in in vivo gene therapy in primates by protecting the retroviral vector particles from lysis by primate complement *in vivo* .

5 It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and preview of this application and scope of the appended claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

- (A) NAME: The United States of America,
as represented by
The Secretary of the Department
of Health and Human Services
- (B) STREET: 6011 Executive Blvd., Suite 325
- (C) CITY: Rockville
- (D) STATE: Maryland
- (E) COUNTRY: U.S.A.
- (F) POSTAL CODE (ZIP): 20852
- (G) TELEPHONE: (301) 496-7056
- (H) TELEFAX: (301) 402-0220
- (I) TELEX:

(ii) TITLE OF INVENTION: PRE-BINDING OF RETROVIRAL VECTOR
PARTICLES WITH COMPLEMENT COMPONENTS TO ENABLE THE
PERFORMANCE OF HUMAN GENE THERAPY IN VIVO

(iii) NUMBER OF SEQUENCES: 2

(v) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

- (A) APPLICATION NUMBER: WO to be assigned
- (B) FILING DATE: July 28, 1993
- (C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

- (A) APPLICATION NUMBER: US 08/098,944
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(viii) ATTORNEY/AGENT INFORMATION:

- (A) NAME: Kruse, Norman J.
- (B) REGISTRATION NUMBER: 35,235
- (C) REFERENCE/DOCKET NUMBER: 15280-138

(ix) TELECOMMUNICATION INFORMATION:

- (A) TELEPHONE: (415) 543-9600
- (B) TELEFAX: (415) 543-5043

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Moloney murine leukemia virus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Ala Val Gln Asp Asp Leu Arg Glu Val Glu Lys Ser Ile Ser Asn Leu
1 5 10 15

Glu Lys Ser Leu
20

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Moloney murine leukemia virus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Arg Asp Ser Met Ala Lys Leu Arg Glu Arg Leu Asn Gln Arg Gln Lys

Leu Phe Glu Ser
20

WHAT IS CLAIMED IS:

1. A method of masking a primate retroviral vector particle from a primate complement system comprising:
 - 5 a) incubating the retroviral vector particle with an isolated primate C1 complement subcomponent in an amount sufficient to block retroviral particle-induced activation of primate C1 complement *in vivo*, wherein said incubating forms a retroviral vector particle:C1 complement subcomponent complex,
10 wherein said retroviral vector particle retains biological activity, and wherein said C1 complement subcomponent is selected from the group consisting of C1s and C1q; and
 - b) administering said complex to a primate to mask
15 the primate retroviral vector particle from the complement system of the primate.
2. The method of claim 1 wherein said C1 complement subcomponent is C1q.
- 20 3. The method of claim 1 wherein said C1 complement subcomponent is C1s.
4. The method of claim 1 wherein said retroviral vector particle is derived from a murine leukemia virus.
25
5. The method of claim 1 wherein said retroviral vector particle contains a heterologous gene operably linked to a portion of the vector which recombines with the genome of a primate.
30
6. A method of masking a primate retroviral vector particle from a primate complement system comprising
 - 35 a) incubating the retroviral vector particle with an antibody fragment in an amount sufficient to block retroviral vector particle-induced activation of primate C1 complement *in vivo*, said antibody fragment capable of binding the retroviral vector particle and lacking a complement binding region, and wherein said incubating forms a retroviral

vector particle:antibody fragment complex and wherein said retroviral vector particle retains biological activity; and

b) administering said complex to a primate.

5 7. The method of claim 6 wherein said antibody fragment is a F_{ab} antibody fragment.

8. The method of claim 6 wherein said antibody fragment is reactive with a viral complement receptor.

10 9. The method of claim 6 wherein said retroviral vector particle is derived from a murine leukemia virus.

15 10. The method of claim 6 wherein said retroviral vector particle contains a heterologous gene operably linked to a portion of the vector which recombines with the genome of a primate.

20 11. A pharmaceutical composition for gene therapy comprising pharmaceutically acceptable excipients and a biologically active primate retroviral vector particle:C1 complement subcomponent complex, said C1 complement subcomponent being present in sufficient amount to block retroviral vector-induced primate C1 complement activation *in vivo*, and said C1 complement subcomponent being selected from

25 the group consisting of C1s and C1q.

30 12. The composition of claim 11 wherein said retroviral vector particle contains a heterologous gene operably linked to a portion of the vector which recombines with the genome of a primate.

35 13. A method of gene therapy comprising administering the composition of claim 11 to a primate.

14. The pharmaceutical composition of claim 11 wherein said C1 complement subcomponent is C1q.

15. The pharmaceutical composition of claim 11 wherein said C1 complement subcomponent is C1s.

5 16. A pharmaceutical composition for gene therapy comprising pharmaceutically acceptable excipients and a biologically active primate retroviral vector particle: C1 complement antibody fragment complex, said antibody fragment being present in sufficient amounts to block retroviral vector-induced primate C1 complement activation *in vivo*, and
10 said antibody fragment capable of binding the retroviral vector particle and lacking a complement binding region.

17. The composition of claim 16 wherein said retroviral vector particle contains a heterologous gene
15 operably linked to a portion of the vector which recombines with the genome of a primate.

18. A method of gene therapy comprising administering the composition of claim 16 to a primate.
20

INTERNATIONAL SEARCH REPORT

Internal Application No
PCT/US 94/08526A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BIOCHEMISTRY, vol.19, no.12, 10 June 1980, WASHINGTON DC, USA pages 2847 - 2853 R. BARTHOLOMEW ET AL. 'Mechanism of antibody-independent activation of the first component of complement (C1) on retrovirus membranes.' cited in the application see abstract --- -/--	1-18

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search

17 November 1994

Date of mailing of the international search report

01.12.94

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Nooij, F

INTERNATIONAL SEARCH REPORT

 Interna 1 Application No
 PCT/US 94/08526

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SCIENCE, vol.260, no.5110, 14 May 1993, WASHINGTON DC, USA pages 926 - 932 R. MULLIGAN 'The basic science of gene therapy.' cited in the application see page 926, right column, line 65 - page 927, left column, line 13 see page 931, left column, line 34 - line 58 ---	1-18
A	NATURE, vol.357, no.6378, 11 June 1992, LONDON, GB pages 455 - 460 A. MILLER 'Human gene therapy comes of age.' cited in the application see the whole document ---	1-18
A	NATURE, vol.293, no.5833, 15 October 1981, LONDON, GB pages 543 - 548 T. SHINNICK ET AL. 'Nucleotide sequence of Moloney murine leukaemia virus.' see figures 1,2 ---	1-18
A	WO,A,92 11359 (UNIV. OF PITTSBURGH OF THE COMMONWEALTH SYSTEM OF HIGHER EDUCATION) 9 July 1992 see claims ---	1-18
P,X	WO,A,93 25698 (THE UNITED STATES GOVERNMENT) 23 December 1993 see the whole document ---	1-18
P,X	WO,A,94 11524 (THE UNITED STATES GOVERNMENT) 26 May 1994 see page 9, line 6 - page 13, line 14 see page 37 - page 39 -----	1-18

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 94/08526

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark : Although claims 1-10,13 and 18 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 94/08526

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9211359	09-07-92	EP-A- 0563239 JP-T- 6504440	06-10-93 26-05-94
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WO-A-9325698	23-12-93	NONE	
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WO-A-9411524	26-05-94	AU-B- 5590194	08-06-94
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